

CLAIMS

1. An antibody comprising a single-chain polypeptide having binding activity against TPO receptor (Mpl), wherein said antibody comprises two heavy chain variable regions and two light chain variable regions.
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2. The antibody of claim 1, wherein the two heavy chain variable regions and the two light chain variable regions are arranged in the order of heavy chain variable region, light chain variable region,
10 heavy chain variable region, and light chain variable region from the N terminus of the single-chain polypeptide.
3. The antibody of claim 1 or 2, wherein the two heavy chain variable regions and the two light chain variable regions are linked by linkers.
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4. The antibody of claim 3, wherein the linkers comprise 15 amino acids.
5. A chimeric antibody that binds to Mpl.
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6. The antibody of claim 5, which is a humanized antibody.
7. The antibody of claim 5 or 6, which is a minibody.
- 25 8. An antibody that binds to soluble Mpl.
9. An antibody that binds to human Mpl and monkey Mpl.
10. An antibody having agonistic activity against human Mpl and monkey
30 Mpl.
11. An antibody whose binding activity to soluble Mpl is $KD = 10^{-6}$ M or lower.
- 35 12. An antibody whose binding activity to soluble Mpl is $KD = 10^{-7}$ M or lower.

13. An antibody whose TPO agonistic activity is $EC_{50} = 100$ nM or lower.

14. An antibody whose TPO agonistic activity is $EC_{50} = 30$ nM or lower.

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15. An antibody whose TPO agonistic activity is $EC_{50} = 10$ nM or lower.

16. An antibody which comprises a heavy chain variable region, wherein said heavy chain variable regions comprises CDR1, CDR2 and CDR3 consisting of an amino acid sequence of any one of:

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[1] SEQ ID NOs: 3, 4, and 5

[2] SEQ ID NOs: 6, 7, and 8

[3] SEQ ID NOs: 9, 10, and 11

[4] SEQ ID NOs: 15, 16, and 17

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[5] SEQ ID NOs: 18, 19, and 20

[6] SEQ ID NOs: 21, 22, and 23

[7] SEQ ID NOs: 24, 25, and 26

[8] SEQ ID NOs: 27, 28, and 29

[9] SEQ ID NOs: 30, 31, and 32

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[10] SEQ ID NOs: 33, 34, and 35

[11] SEQ ID NOs: 36, 37, and 38

[12] SEQ ID NOs: 39, 40, and 41

[13] SEQ ID NOs: 42, 43, and 44

[14] SEQ ID NOs: 48, 49, and 50

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[15] SEQ ID NOs: 51, 52, and 53

[16] SEQ ID NOs: 54, 55, and 56

[17] SEQ ID NOs: 57, 58, and 59.

17. An antibody which comprises a light chain variable region, wherein said light chain variable region comprises CDR1, CDR2 and CDR3 consisting of an amino acid sequence of any one of:

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[1] SEQ ID NOs: 60, 61, and 62

[2] SEQ ID NOs: 63, 64, and 65

[3] SEQ ID NOs: 78, 79, and 80

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[4] SEQ ID NOs: 84, 85, and 86

[5] SEQ ID NOs: 93, 94, and 95

[6] SEQ ID NOs: 96, 97, and 98

[7] SEQ ID NOs: 102, 103, and 104

[8] SEQ ID NOs: 108, 109, and 110

[9] SEQ ID NOs: 111, 112, and 113

5 [10] SEQ ID NOs: 114, 115, and 116.

18. An antibody that comprises a heavy chain variable region and a light chain variable region of any one of:

10 [1] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 3, 4, and 5, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 60, 61, and 62;

15 [2] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 6, 7, and 8, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

20 [3] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 9, 10, and 11, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

25 [4] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 15, 16, and 17, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

30 [5] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 18, 19, and 20, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

35 [6] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 21, 22, and 23, and a light chain variable region that comprises

CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 78, 79, and 80;

5 [7] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 24, 25, and 26, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

10 [8] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 27, 28, and 29, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 84, 85, and 86;

15 [9] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 30, 31, and 32, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequence consisting of SEQ ID NOs: 63, 64, and 65;

20 [10] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 33, 34, and 35, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

25 [11] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 36, 37, and 38, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 93, 94, and 95;

30 [12] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 39, 40, and 41, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 96, 97, and 98;

35 [13] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 42, 43, and 44, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting

of SEQ ID NOs: 78, 79, and 80;

[14] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 45, 46, and 47, and a light chain variable region that comprises
5 CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 102, 103, and 104;

[15] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 48, 49, and 50, and a light chain variable region that comprises
10 CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[16] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 51, 52, and 53, and a light chain variable region that comprises
15 CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 108, 109, and 110,

[17] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 54, 55, and 56, and a light chain variable region that comprises
20 CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 111, 112, and 113;

[18] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 57, 58, and 59, and a light chain variable region that comprises
25 CDR1, CDR2, and CDR3 each comprising the amino acid sequences consisting of SEQ ID NOs: 114, 115, and 116.

19. An antibody that comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 118.

30 20. An antibody that comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 120.

21. An antibody that comprises a heavy chain variable region
35 comprising the amino acid sequence of SEQ ID NO: 118 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 120.

22. An antibody comprising the amino acid sequence of SEQ ID NO: 122 or 264.

5 23. An antibody that comprises a heavy chain variable region, wherein said heavy chain variable region comprises FR1, FR2, FR3, and FR4 consisting of amino acid sequences of any one of:

[1] SEQ ID NOs: 230, 232, 234, and 236

[2] SEQ ID NOs: 265, 267, 269, and 271

10 [3] SEQ ID NOs: 279, 281, 283, and 285

[4] SEQ ID NOs: 298, 299, 300, and 301

[5] SEQ ID NOs: 298, 299, 306, and 301.

24. An antibody comprising a light chain variable region, wherein
15 said light chain variable region comprises FR1, FR2, FR3, and FR4 consisting of amino acid sequences of any one of:

[1] SEQ ID NOs: 239, 241, 243, and 245

[2] SEQ ID NOs: 272, 274, 276, and 278

[3] SEQ ID NOs: 302, 303, 304, and 305

20 [4] SEQ ID NOs: 302, 307, 308, and 305.

25. An antibody that comprises a heavy chain variable region and a light chain variable region of any one of:

[1] a heavy chain variable region which comprises FR1, FR2, FR3,
25 and FR4 having the amino acid sequences consisting of SEQ ID NOs: 230, 232, 234, and 236, and a light chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 239, 241, 243, and 245;

[2] a heavy chain variable region which comprises FR1, FR2, FR3,
30 and FR4 having the amino acid sequences consisting of SEQ ID NOs: 265, 267, 269, and 271, and a light chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 272, 274, 276, and 278;

[3] a heavy chain variable region which comprises FR1, FR2, FR3,
35 and FR4 having the amino acid sequences consisting of SEQ ID NOs: 279, 281, 283, and 285, and a light chain variable region which

comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 272, 274, 276, and 278;

[4] a heavy chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 298, 299, 300, and 301, and a light chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 302, 303, 304, and 305;

[5] a heavy chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 298, 299, 306, and 301, and a light chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 302, 307, 308, and 305.

26. An antibody that comprises a heavy chain variable region, wherein said heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 229, 256, 262, 289, or 295.

27. An antibody that comprises a light chain variable region, wherein said light chain variable region comprises the amino acid sequence of SEQ ID NO: 238, 258, 291, or 297.

28. An antibody that comprises a heavy chain variable region and a light chain variable region of any one of:

[1] a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 229, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 238;

[2] a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 256, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 258;

[3] a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 262, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 258;

[4] a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 289, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 291;

[5] a heavy chain variable region comprising the amino acid

sequence of SEQ ID NO: 295, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 297.

- 5 29. An antibody that comprises the amino acid sequence of SEQ ID NO: 2, 254, 260, 287, or 293.
- 10 30. An antibody having an activity equivalent to that of an antibody of any one of claims 16 to 29, wherein said antibody comprises the amino acid sequence set forth in any one of claims 16 to 29, in which one or more amino acids have been substituted, deleted, added and/or inserted.
- 15 31. An antibody that recognizes an epitope recognized by an antibody of any one of claims 16 to 30.
32. An antibody that recognizes the region of amino acids 26 to 274 of human Mpl.
- 20 33. An antibody of any one of claims 1 to 32, which has TPO agonistic activity.
34. A polynucleotide encoding an antibody of any one of claims 1 to 33.
- 25 35. A polynucleotide hybridizing to the polynucleotide of claim 34 under stringent conditions, wherein said polynucleotide encodes an antibody having activity equivalent to that of an antibody of any one of claims 1 to 33.
- 30 36. A vector comprising the polynucleotide of claim 34 or 35.
37. A host cell that carries the polynucleotide of claim 34 or 35, or the vector of claim 36.
- 35 38. A pharmaceutical composition comprising an antibody of any one of claims 1 to 33.